#4457

CAR19 T Cells Redirected to Novel Antigens Mediate Robust Cytotoxicity Against Diverse Malignancies

Paul D. Rennert[1], Lihe Su[1], Fay Dufort[1], Lan Wu[1], Andreas Hombach[2], Hinrich Abken[2],Roy Lobb[1] and Christine Ambrose[1]
1 - Aleta Biotherapeutics, Natick, MA; 2 - University Of Cologne, Cologne, Germany

1 - Introduction

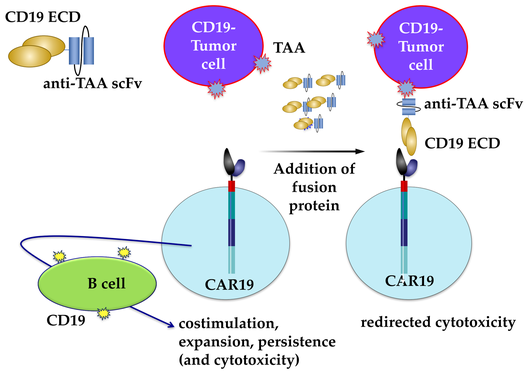
Adoptive cellular therapy can cure advanced B cell leukemias and lymphomas. This progress has been made using CARs that recognize B cell antigens, particularly CD19, referred to here as CAR19 T cells. One component of the unique success of CAR19 adoptive cellular therapy is the provision of antigen and productive costimulation by normal CD19+ B cells in protected niches, and exiting the bone marrow post-lymphodepletion, allowing the CAR19 T cell population to persist for many months or even longer. However, an emerging issue in the treatment of advanced B cell malignancies with CAR19 cellular therapeutics is loss of CD19 antigen on the target tumor cells, and subsequent patient relapse. Further, and in contrast with CD19+ B cell malignancies, progress against other cancers has been limited.

Here we present a novel strategy to leverage the potency and persistence of CAR19 T cells by redirecting their cytotoxic activity to novel tumor antigens. The technology, termed IMPACT^{tm} (**I**ntegrated **M**odular **P**roteins for **A**doptive **C**ell **T**herapy) can be applied to diverse antigens and tumor types. We present therapeutic modalities that address the issue of antigen loss in B cell leukemias & lymphomas, and that enable the targeting of diverse antigens in CLL, AML and multiple myeloma.

2 - Technology Overview

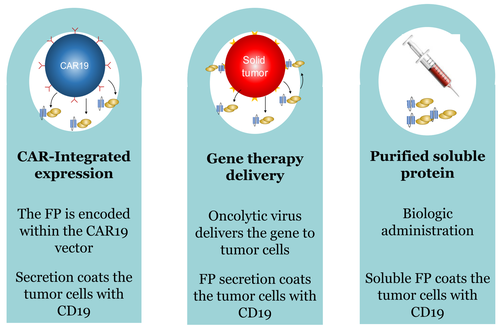
IMPACT fusion proteins (FP) are created by cloning the extracellular domain (ECD) of a CAR T cell target protein (e.g. CD19) to an scFv that recognizes a tumor associated antigen (TAA). The system is modular: diverse ECD-scFv fusion proteins have been designed and expressed. The FP create a bridge between the CAR19 T cell and the target antigen+ cell (Figure 1)

Figure 1. Bridging CAR19 T cells to antigen+ tumor cells



3 – Delivery of FP to the target tumor

We have developed three distinct methods to deliver FP to the tumor microenvironment.



4 – FP Activity

Here we use soluble FP to investigate the biologic properties inherent in the system, a prerequisite to developing the CAR-T integrated FP modules as therapeutics. FP affinities for targets (anti-CD19//CD19-ECD; anti-TAA//TAA) are measured in ELISA and flow cytometry assays, and the potency of the FP is assessed in cytotoxicity assays (Figures 2, 3).

Figure 2. Affinity of the CD19-anti-CD20 FP for 293-CD20 cells, as detected with anti-CD19-fluorescent antibody (FMC63-PE)

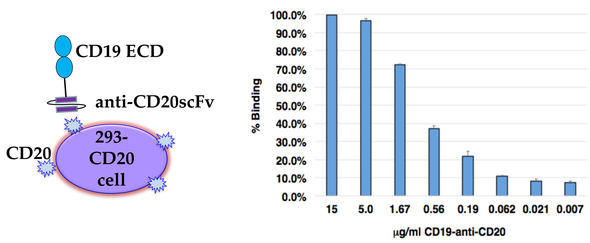


Figure 3. CD19-anti-CD20 FP-mediated dose responsive killing of 293-CD20 cells by redirected CAR19 T cells

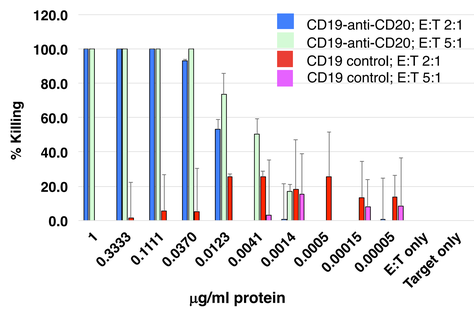
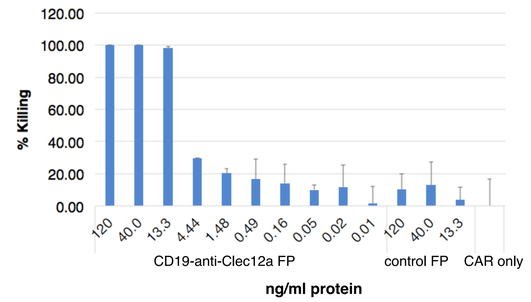
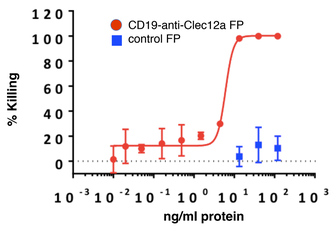


Figure 4. CD19-anti-Clec21a FP-mediated dose responsive killing of U937 AML cells by redirected CAR19 T cells



Similar analyses have been performed on all Aleta FPs. This has led to the finding that a) potency correlates with affinity and b) potency is much greater than affinity (Figure 5 and Table 1).

Figure 5. Curve fitting analysis of cytotoxicity reveals potency at pM concentration.



KEY POINTS

- activity at very low concentration
- no inactivation in the presence of excess fusion protein up to 15µg/ml

Table 1. Potency analysis of diverse FP therapeutics.

Purified FPs were used to redirect CAR19 T cells to diverse tumor antigens on the indicated cell lines. E:T ratio was 5:1 using a CAR19 prep with 50% transduction efficiency. In all cases the EC_{50} for cytotoxicity was at least 1 log improved over the EC_{50} for cell binding. Cell binding affinities of 1-70 nM support pM cytotoxicity.

FP or biFP	binding EC_{50} (FACS)	cytotoxicity EC_{50}	target cell	fold change
CD19-anti-CD20	20nM	150pM	293-CD20	133x
CD19-anti-BCMA	70nM	750pM	H929 MM	93x
CD19-anti-Clec12a	20nM	100pm	U937 AML	200x
CD19-anti-ROR1	4nM	220pM	293-ROR1	18.5x
CD19-anti-ROR1	1nM	50pM	786-O RCC	20x
CD19-anti-Her2	2nM	10pM	SKOV3 OvCa	200x
CD19-anti-Her2-anti-EGFR	2nM (Her2)	0.7pM (Her2)	SKOV3 (Her2+/EGFR-)	~2800x
CD19-anti-Her2-anti-EGFR	4nm (EGFR)	0.8pM (EGFR)	A431 (Her2-/EGFR+)	5000x

5 - CAR19 with the IMPACT^{TM} FP encoded as an integrated gene

The development candidates are being constructed as integrated genes (i-gene FP) using lentiviral vectors and packaging systems. A prototype schematic is shown here:

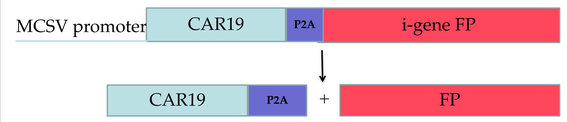


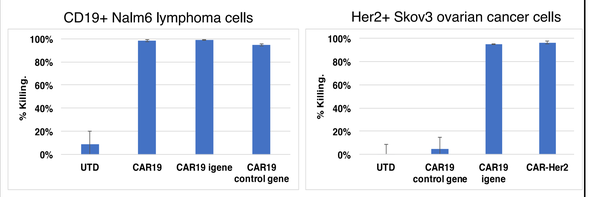
Table 2. Programs in development.

We use Lentigen's vector systems that are optimized for expression in primary T cells. The following CAR19-i-gene FP constructs are under development:

i-gene FP	status	transduction %	FP secretion	indications
CD19-anti-Her2	primary T cells	> 50	20-40 ng/ml	solid tumors
CD19-anti-CD20	SupT transduction	> 50	> 50 ng/ml	ALL, NHL
CD19-anti-BCMA	virus scale up	na	na	MM
CD19-anti-ROR1	virus scale up	na	na	CLL, solid tumors
CD19-anti-Clec12a	viral vector cloning	na	na	AML
CD19-anti-BCMA-anti-CD38	construct cloning	na	na	MM

6 – Serial cytotoxicity

Figure 6. PoC using the CAR19 T cell with the Her2 igene



The degree of cytotoxicity observed is similar regardless of the "direction" of serial killing ie. Nalm > Skov or Skov > Nalm. Preliminary data indicate that engagement of Nalm (a CD19+ B cell) provides important costimulatory signals and improves the phenotype of the CAR19 T cell, with properties consistent with robust expansion and persistence (preliminary data not shown).

7 - Conclusions and path forward

IMPACT^{TM} fusion proteins mediate redirected tumor cell killing and can be successfully secreted from CAR T cells well in excess of the concentration required for cytotoxiciy. The first *in vivo* studies using CAR19 cells carrying specific i-gene FP cassettes are in life.